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Natural Cytotoxicity in Cancer Patients

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INTRODUCTION

It has been suggested that natural cytotoxicity may play an important role in immune serveillance against tumors in experimental animals and in humans (1, 10). In mice, natural killer (NK) activity has been shown to vary according to the strain and age of the mice tested, and to several environmental factors (11). Tumor bearing mice have been found to have depressed levels of NK activity (18) and indirect evidences are accumulating that NK cells play some role in tumor rejection in vivo (14, 24). In man, however, there have been several reports on NK activity in cancer patients (3, 9, 13, 20) but the results are still controversial.

Therefore, it seems worthwhile to ascertain whether there exists any correlation of NK activity with the clinical course and to elucidate the factors affecting levels in NK activity in cancer patients.

MATERIALS AND METHOD

1. Clinical materials

Heparinized venous blood was collected from patients of various stages of gastric carcinoma that were admitted at our hospital. The control samples were donated by healthy adult volunteers. Sera from normal volunteers and patients with acute hepatitis and cancers of the stomach, the pancreas and the liver were collected and stored in a freezer until the serum effect on NK activity was assayed.

2. Lymphocyte preparation and cytotoxicity assay (Fig. 1)

Heparinized whole blood from normal donors and patients with gastric cancer were separated by centrifugation on discontinuous ficoll-isopaque gradients (6).

The interface, rich in mononuclear cells, was washed with a balanced salt solution and adjusted to the appropriate cell concentration in RPMI 1640 containing ten percent fetal calf serum. The in vitro ^{51}Cr release cytotoxicity assay (15), using K562 cells as target cells, was undertaken between 5×10^5 of these peripheral mononuclear cells and 1×10^4 of ^{51}Cr labeled target cells for four hours. After incubation, the cell free supernatant of each culture was counted in a LKB Wallac

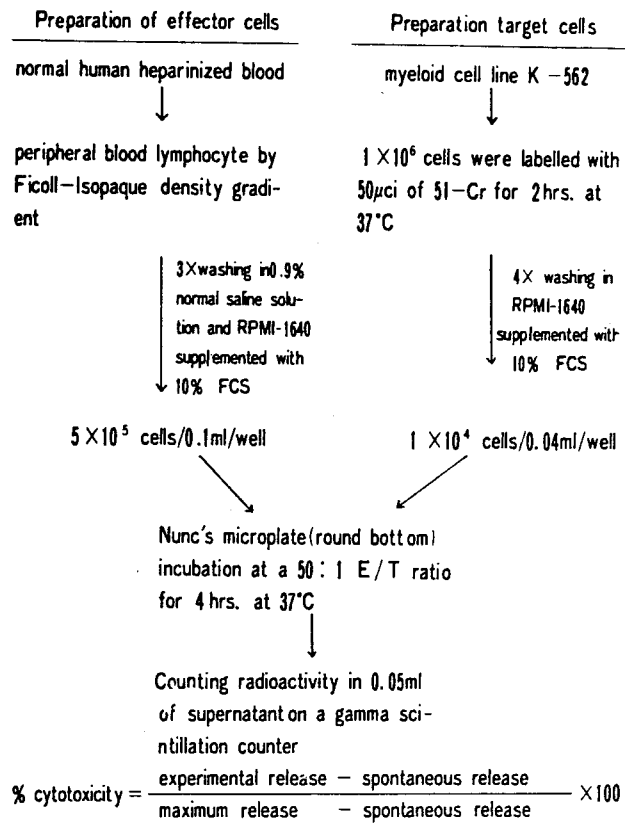

Fig. 1. Procedure of ^{51}Cr release assay for natural cytotoxicity.

autogamma scintillation counter to determine the released ^{51}Cr and the percent cytotoxicity was calculated by the following formula;

$$\% \text{ cytotoxicity} = \frac{\text{lymphocyte mediated release} - \text{spontaneous release}}{\text{maximum release} - \text{spontaneous release}} \times 100$$

Each assay was done in triplicate and the statistical difference between sets of data were tested using the student's t test.

RESULTS

1. NK activity of normal donors and patients with gastric cancer

NK activity was tested in 15 normal donors and 18 patients with gastric cancer prior to the treatment. The mean percent cytotoxicity of normal donors was $51.3 \pm 12.3\%$ and that of patients with gastric cancer was $27.1 \pm 12.6\%$.

In comparing the results of patients with normal donors, a significant difference ($p < 0.01$) was noted in patient group having lower activities as shown in Fig. 2.

2. Relationship between NK activity and stage of gastric cancer

Patients with untreated gastric cancer were classified according to the diseased stage as shown in Fig. 3. The mean percent cytotoxicity of five patients with early gastric cancer (stage I) was $44.2 \pm 6.9\%$ and was not siginificantly different from that of the normal donors. On the other hand, in advanced stages (II, III, and IV) of gastric cancer, the mean % cytotoxicity was lower than that of normal donors ($p < 0.01$ in stage II, III, and $p < 0.001$ in stage IV). The weakest cytotoxicity was observed in the terminal (IV) stage of the disease.

3. Effect of treatment on NK activity

The NK activity was compared in preoperative as well as postoperative patients two months after gastrectomy of four early gastric cancer and three resectable advanced cancers as shown in Fig. 4. In one out of three advanced cancer, in which the tumor was completely resected, a remarkable improvement of % cytotoxicity from 20 to 40% was observed and even in four early gastric cancer patients, whose mean % cytotoxicity was not significantly different from that of normal donors, two of them showed higher levels of NK activity after the operation.

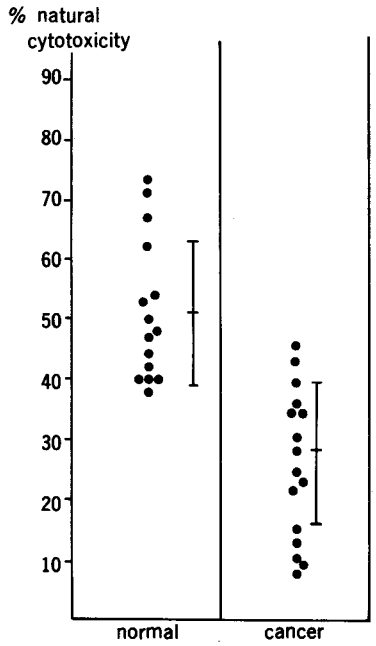

Fig. 2. Natural cytotoxicity of lymphocytes from normal donors and patients with gastric cancer

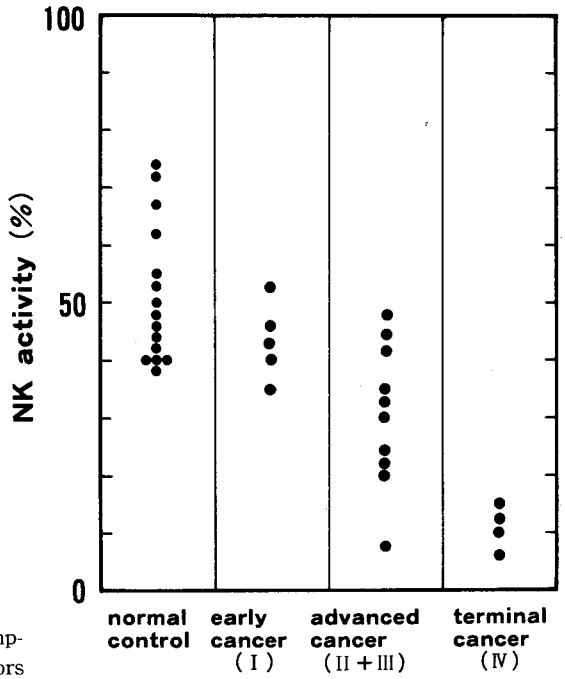

Fig. 3. Relationship between NK activity and stage of gastric cancer.

The effect of chemotherapy in the group of non-operable patients was tested and the results are shown in Fig. 5. Nine of advanced gastric cancer patients were treated with 5-fluorouracil and mitomycin C and six of them were less reactive after the chemotherapy, suggesting that chemotherapy itself may be a suppressive agent for NK activity. However, as shown in open circle in Fig. 5, three out of them showed elevation of % cytotoxicity, which responded well to anticancerous chemotherapy. On the other hand, poor responders against chemotherapy showed no change or depressed activity of natural cytotoxicity as shown in closed circle in Fig. 5. Serial changes of NK activity from each of typical responder and poor responder against chemotherapy was compared in Fig. 6. In a non-responding case against chemotherapy, NK activity decreased as the disease progressed. However, NK activity of responding patients against chemotherapy maintained higher values as compared with the value prior the treatment.

4. *Effect of sera from normal donors and cancer patients against NK activity*

Sera from normal donors and patients with tumor of the stomach, liver,

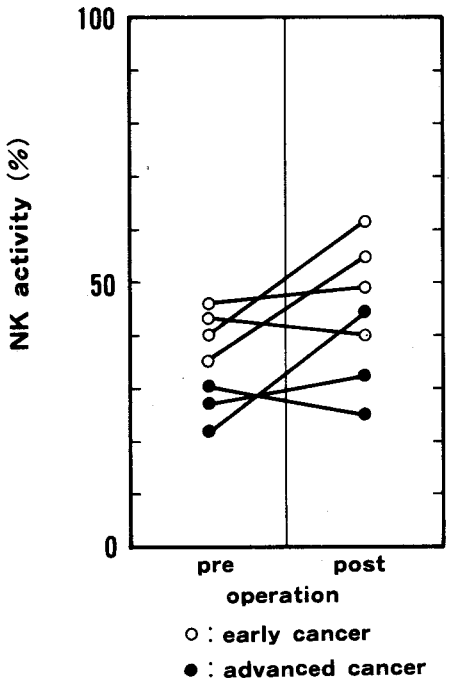

Fig. 4. Effect of surgical removal of tumor on natural cytotoxicity in patients with gastric cancer.

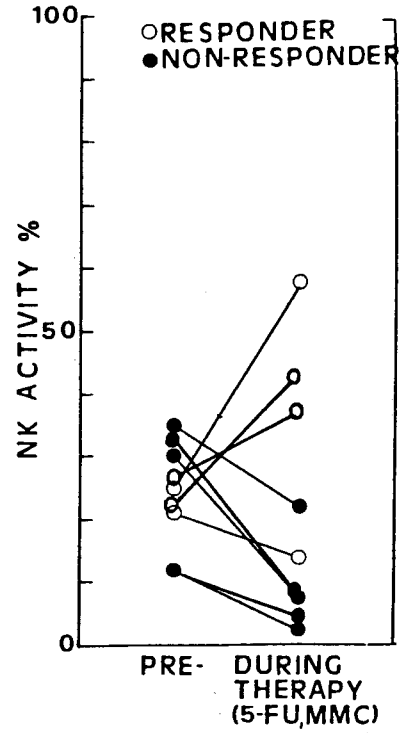

Fig. 5. Effect of anticancerous chemotherapy on natural cytotoxicity in patients with advanced gastric cancer.

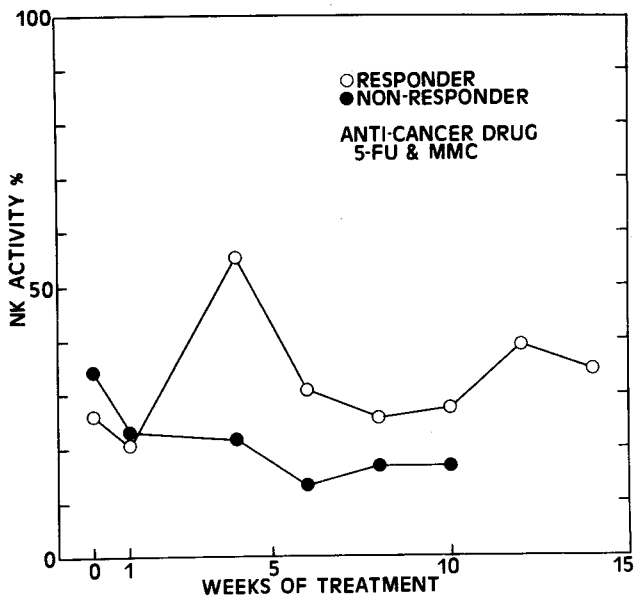

Fig. 6. Serial change of natural cytotoxicity of patients with gastric cancer during chemotherapy.

pancreas and with acute hepatitis were collected and the suppressive activity of sera was determined. Assays were made with an addition of cancerous serum at a final concentration of 10 percent in cytotoxic assay using normal lymphocytes against K562 target cells. The effect of sera from cancer patients on NK activity are shown in Fig. 7. Although the sera from normal subjects and acute hepatitis patients did not affect NK activity, the mean % cytotoxicity was $36.2 \pm 11.1\%$ in gastric cancer, $33.5 \pm 8.8\%$ in hepatoma, and $37.3 \pm 6.1\%$ in pancreatic cancer. These results showed significant suppression of NK activity by sera from cancer patients.

DISCUSSION

Natural killer cells are members of cytotoxic cells which react aginst tumor cells and certain other types of target cells (1). They appear to be a small subpopulation of T-lymphocyte lineage which were found to have low affinity receptors for erythrocytes (E) (22). In mice and rats, natural cytotoxicity has been described regarding various allogeneic and syngeneic tumor cells (11, 18) and a possible defense mechanism of this cytotoxicity for immune serveillance against tumors was suggested by Zahring (24) and Herberman (10). In spite of these impressive documentations in animal experiments, the biological significance and actual levels of natural cytotoxicity in man is not clear. Pross and Baines (14)

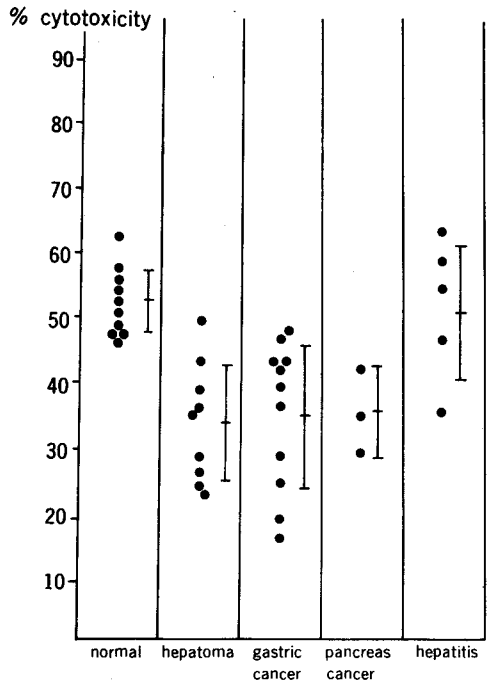

Fig. 7. Effect of sera from normal donors and patients with various cancer of digestive organs against natural cytotoxicity.

first reported the redution of natural cytotoxicity in patients with metastatic carcinoma and patients with untreated chronic lymphocytic leukemia, whereas no significant difference was found between healthy controls and patients with clinically localized malignancy of many different histological types. Takasugi et al (20) found a similar impairment of natural cytotoxicity with increasing tumor involvement. Becker et al (2) also found reduced natural cytotoxicity in tumor bearing rats. Ermin (8), however, could not find any relationship between healthy donors and patients with various malignancies. In our present studies, patients with gastric carcinoma were chosen and classified according to the stage of the disease to clarify such contoversial results of NK activity in tumor bearers. NK activity in patients with gastric cancer prior to the treatment were found to be depressed in almost all stages of the disease except early gastric cancer, which results were essentialy similar to that of Pross and Bainess (14). Furthermore, the decline of NK activity was observed with the increment of the tumor burden. The effect of treatment of NK cell activity is another matter. Our studies have shown that cytoreductive surgery was effective in the recovery of the impaired NK activity not

only in advanced non-resectable cancer but even in certain cases of early gastric cancer, indicating that the reduction of tumor burden is an important factor to improve natural cytotoxicity. This phenomenon is also supported by the results of effective anticancerous chemotherapy in patient with advanced gastric cancer. The group that responded well to chemotherapy showed a recovery of natural cytotoxicity whereas NK activity in nonresponder remained low. Saijo (17) found similar results and suggested that NK activity seemed to be a more reliable parameter in lymphocyte immune response than PHA-blastogenesis for the evaluation of the effect of chemotherapy. For practical purposes in man, it is important to distinguish whether the weaker reactivity allows tumor occurence or whether invasion or the presence of the tumor results in a weaker reactivity (4, 5, 7, 19). In the present study, the latter possibility is more likely because no signigicant difference of NK activity between healthy controls and early gastric cancer patients was observed. Despite of the fact that the depression of NK activity in malignancy is thus clinically evident, the mechanism responsible for it is still unclarified. Hochman and Gudkowicz (12) and Savary and Lottzoa (16) reported the role of suppressor cells against natural killer cells in mice treated with C. parvum and hydrocortisone. In addition to such suppressor cells of animal experiments, our present data demontrated that sera from cancer patients appears to contain factor (s) which is capable of inhibiting natural cytotoxicity. These humoral inhibitions on immune reaction have been reported in other proliferative responses of lymphocytes, e. g. blastogenesis to mitogens, and allogenic stimulation (23, 24).

The humoral inhibitions of NK activity may be a reflection of an in vivo reduction of cancer patient immunity and our current studies have centered on the purification and characterization of this serum factor.

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